CHAPTER 1

INTRODUCTION

1.1 Principles, Theory, Rationale and/or Hypotheses

The *Stemona* plant is known in the Thai vernacular as "Non Tai Yak". The extracts of *Stemona* roots have been used in traditional medicine in South East Asia, China and Japan to treat the symptoms of bronchitis, pertussis and tuberculosis and also have been used as antiparasitic agent on humans and animals (Greger, 2006.). Mungkornasawakul *et al.* (2003 and 2004) reported the isolation of two new *Stemona* alkaloids, stemocurtisine and stemocurtisinol along with oxyprotostemonine from the root extracts of *Stemona curtisii* with the investigation of the larvicidal activity on mosquito larvae (*Anopheles minimus*). It was found that the most potent compound was oxyprotostemonine. The crude extract of this plant has been formulated into a "biopesticide" which shows great potential in agricultural field trials as an effective "natural pesticide" (Sastraruji, 2006). Moreover, unidentified *Stemona* species from Lampang Province, Northern Thailand had been reported the isolation of 1′, 2′-didehydrostemofoline (Sastraruji *et al.*, 2005). In 2009, Baird *et al.* reported that 1′,2′-didehydrostemofoline has shown the highest inhibitory activity of acetylcholinesterase which indicated its potential for the treatment of Alzheimer's disease.

Propagation of these plants through seeds has so far been unreliable due to poor germination. Furthermore, the plants would need at least 3-5 years to produce sufficient alkaloid contents suitable for harvesting. The alkaloid contents are variable and at times were found to be low depending on various conditions such as genetics and environmental factors. In addition, harvesting the plant on a mass scale from

natural habitats (Southern region of Thailand) would result in the depletion of the natural resources. Therefore, there is an urgent need to develop a better cultivating technique either by developing a method to overcome the seed dormancy, hence improving seed germination, or by micropropagation (Chotikadachanarong *et al.*, 2005). The production of important alkaloids through different biotechnological means is an innovative approach to large scale alkaloids production, since it would guarantee continuous supply of plant materials, independent of season, soil conditions and other factors which influence the plant growth. The use of root cultures technique is one of the solutions to circumvent these problems. Furthermore, the use of elicitors, precursors and cultures condition will also be incorporated into this approach as an effective strategy to increase the production of important alkaloids in cell and organ cultures (Pitta-Alvarez *et al.*, 2000 and Spollansky *et al.*, 2000).

The aims of this work was to optimize conditions for effective stimulation of the production of *Stemona* alkaloids (1′, 2′-didehydrostemofoline, stemocurtisine, stemocurtisinol and oxyprotostemonine) in root cultures of *Stemona* spp. and improve the production of *Stemona* alkaloids by the approaches described above.

1.2 Research Objectives

1.2.1 To study a time profile of *Stemona* alkaloid (oxyprotostemonine, stemocurtisine and stemocurtisinol) formation in *S. curtisii* root cultures.

1.2.2 To study a time profile of 1′,2′-didehydrostemofoline formation from *Stemona* sp. root cultures.

1.2.3 To study the effects of precursors (sucrose, sodium acetate and tyrosine) on *Stemona* alkaloid production in *S. curtisii* root cultures.

1.2.4 To study the effects of elicitors (salicylic acid, methyl jasmonate, yeast extract and chitosan) on *Stemona* alkaloid production in *S. curtisii* root cultures.

1.2.5 To study the effects of culture conditions (culture temperature, media pH and illumination) on *Stemona* alkaloid production in *S. curtisii* root cultures.